FREE FATTY ACIDS OF THE SKIN SURFACE AND BARRIER ZONE IN NORMAL AND ABNORMAL KERATINIZATION*

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In previous work we have shown that the concentration of free fatty acids in the lipids [*i.e.*, the acid number] from the barrier zone is higher in psoriatic subjects than in normal healthy individuals (1, 2, 3). These studies have now been extended and the nature of these free fatty acids has been determined using the technic of gas chromatography.

MATERIALS AND METHODS

Lipid samples: Samples of the surface and barrier zone lipids were obtained by the methods described in previous publications (4, 5).

Isolation of the free fatty acids and methylation: The lipid sample from a single test site was dissolved in ether and the free fatty acids extracted by prolonged stirring with 1% aqueous sodium carbonate solution. The sodium carbonate solution was separated from the ether layer and washed with further portions of ether. The ether layer and washings contained the neutral fat which was not further investigated. The sodium carbonate extract was acidified with 10% HCl and the liberated free fatty acids extracted with ether. The free fatty acids were recovered from the ether extract by evaporation under nitrogen and then methylated using boron trifluoride (6).

Gas chromatography of the methylated fatty acids: The Chromalab [Glowall Corporation, Glenside, Pa.] gas chromatograph was used in these studies. A six foot coiled glass column, packed with 2.5% SE 30 [General Electric, Silicones Products Department] on Gas Chrom P, 100–140 mesh [Applied Science Laboratories, Inc., State College, Pa.] was used. The Gas Chrom P was pre-treated, silanized with dichlorodimethylsilane and coated as described by Haahti (7) and the column conditioned at 300° C for at least 18 hours prior to use. It was tested for efficiency and absence of tailing by using a standard mixture of cholestane, cholesterol, stigmasterol and sitosterol and discarded if not acceptable. An argon ionization type of detector was used, it was operated at 1000 volts giving a narrow linearity range and a suitable correction factor was applied for responses just above the linear range. Larger samples were suitably diluted to bring the response of all peaks into the linear range. The flash heater was maintained at a temperature of 300° C and the detector oven at 275° C; a flow rate of approximately 100 ml/min. was achieved by an applied pressure of 30 psi. The column was held at a temperature of 100°C until thermal equilibrium was attained, it was then programmed with a temperature increase of 3°/min. The fatty acid sample, dissolved in 1 μl of chloroform, was injected the moment the column temperature reached 125° and the programming continued until a temperature of 310° had been reached. Peaks were identified by reference to known compounds run under identical conditions and, when doubtful, by means of internal standards. On this type of column the sequence of emergence of the peaks is similar to that which occurs with Apieson M or L, in other words, the branched and unsaturated acids emerge ahead of the corresponding saturated. The method of peak designation used in other publications was used (8), for a given chain length the normal straight-chain saturated acid was designated (a) and the branched and unsaturated acids preceding it as (b), (c), etc., thus C_{16b} would denote a mixture of the monounsaturated and singly branched C_{16} acids. No attempt was made in this study to estimate the branched and unsaturated acids separately. The calculation of the percentage composition was performed using the method of Horning, Karmen and Sweely (9).

RESULTS

In previous studies only acids up to a chain length of C_{18} or slightly higher have been studied. By using the technic of temperature programming we have been able to study the acids up to chain lengths of C_{30} and even higher. The acids of longer chain length than C_{18} have proved to be particularly significant as shown in Figs. 1 to 4. Figs. 1 and 2 show the gas chromatograms of the free fatty acids of the surface and barrier zone lipids of a normal healthy individual, while

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This work has been supported by Grants # AM 07137-03 GM and AM 06801-01 GM from the Public Health Service.

Presented at the Twenty-fourth Annual Meeting of The Society for Investigative Dermatology, Inc., Atlantic City, N.J., June 17, 1963.

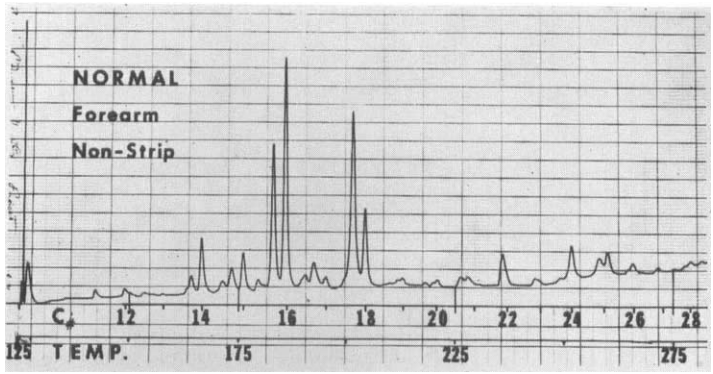

FIG. 1. Gas chromatogram of the methyl esters of the free fatty acids collected from a 20 cm.^{2} area of the flexor surface of the right forearm of a healthy subject. Full scale recorder response 1 $\times$ 10^{-8} amps. For details see text.

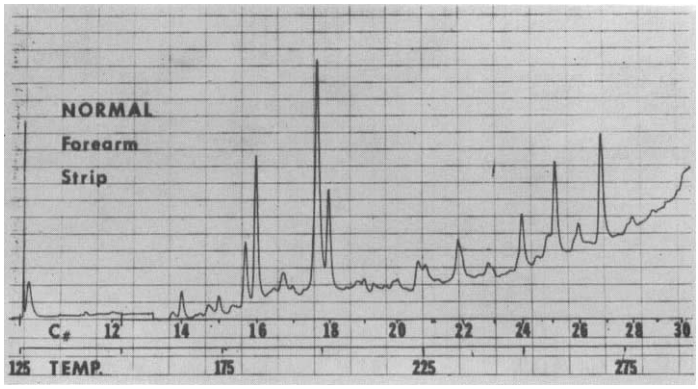

FIG. 2. Gas chromatogram of the free fatty acids collected from the barrier zone level of a stripped site on the left forearm of the same healthy subject as Fig. 1. Full scale recorder response 3 $\times$ 10^{-9} amps.

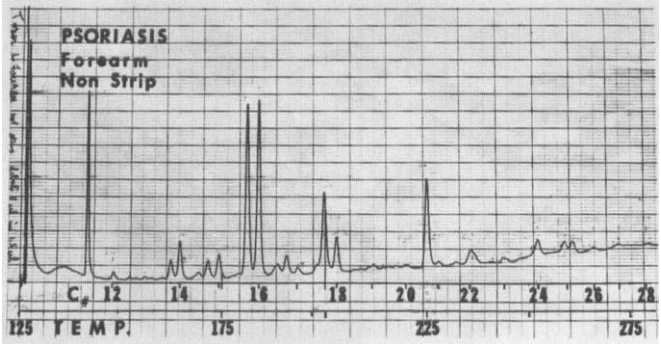

FIG. 3. Gas chromatogram of the free fatty acids from the surface lipids of the forearm of a patient with psoriasis. The test site was clinically uninvolved but psoriatic lesions were in close proximity. Full scale recorder response 1 $\times$ 10^{-9} amps.

Figs. 3 and 4 show the corresponding acids from a psoriatic individual. In all the psoriatic subjects studied the lipid specimens were collected from uninvolved areas of skin. The free fatty acids from the surface lipids of the normal healthy subjects (Fig. 1) show the presence of relatively few of the longer chain-length acids, while the corresponding material from the barrier zone of

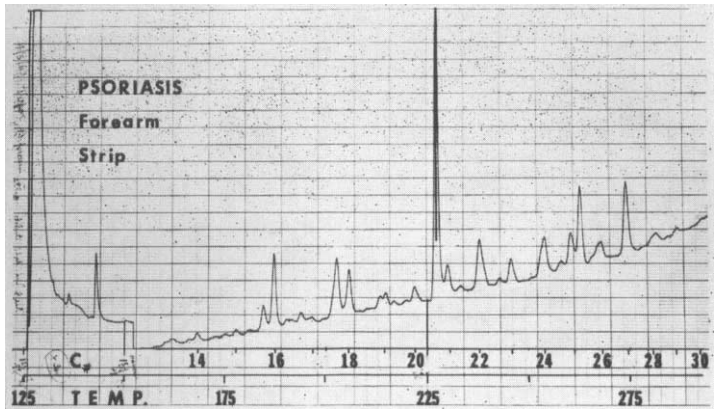

FIG. 4. Gas chromatogram of the free fatty acids from the barrier zone site of the opposite forearm to that used for Fig. 3. Full scale recorder response 3×10^{-9} amps.

the same subject shows a much higher content of these longer acids, in particular, there are relatively large amounts of acids of chain length C_{24} to C_{27} (Fig. 2). The psoriatic subject, on the other hand, shows the presence of appreciable amounts of the longer chain acids both on the surface (Fig. 3) and at the barrier zone (Fig. 4), in particular, a relatively high C_{21b} peak occurs in both chromatograms. The calculated percentage composition from these four chromatograms is shown in Table I.

Considerable variation in the chromatographic pattern of these longer chain acids has been observed both in eleven healthy and in nine psoriatic individuals, but at this stage of our investigations the nature and significance of these individual differences has not been studied. To simplify our study, the percentage of acids of chain length longer than C_{18} has been calculated for each specimen. These results have been summarized in Table II. These results show that in the lipids from the surface of the chest of a healthy subject only an average of 16% are of chain lengths longer than C_{18} compared with an average of 31% for the barrier zone lipids. For the psoriatic subjects the corresponding figures for the surface and barrier zone lipids respectively are 35% and 49%. Thus, there is a significant increase in the longer chain acids both on the surface and at the barrier zone of the clinically uninvolved skin of the psoriatic. Similar, though less significant, changes have been observed for the corresponding lipids from the forearm of these two groups of subjects. In both cases, the percentage of longer chain acids of the surface lipids of the psoriatic subjects approaches that of the barrier zone of the normal group.

In view of the higher concentration of these longer chain acids in the barrier zone lipids it was thought that these particular acids were being formed in the epidermis rather than the sebaceous glands. In order to test this hypothesis a sample of relatively uncontaminated sebum was collected by expressing the large sebaceous glands at the side of the nose of a healthy individual after first wiping the skin surface with re-distilled acetone. A sample of lipids was also obtained from the heat-separated epidermis (10) of abdominal skin from a cadaver. The free fatty acids were isolated from both specimens, methylated and analysed by gas chromatography. The gas chromatograms of these two analyses are shown in Figs. 5 and 6. The free fatty acids from the sebum (Fig. 5) shows the presence of almost negligible amounts of the longer chain acids while the epidermal lipids contained large amounts of these acids (Fig. 6). There is thus some evidence for the view that these longer chain acids are being formed in the epidermis at or near the barrier zone.

DISCUSSION

Investigation of the free fatty acids from the lipids of the skin surface and barrier zone of normal healthy and psoriatic individuals has revealed some significant results. While previous investigators have not studied the acids of chain length higher than C_{20}, we have been able to study the longer chain acids up to a chain length of C_{30} and the changes described in this investiga-

TABLE I

Composition of the Free Fatty Acids from the Lipids of the Surface and Barrier Zone of the Forearm of a Healthy Subject and a Psoriatic Subject

	Healthy		Psoriatic	
	Surface	Barrier	Surface	Barrier
	%	%	%	%
Below C_{14}	1.7	.6	1.7	1.1
C_{14} c	.7	—	.1	.2
b	1.4	.5	2.0	.3
a	4.3	1.9	3.6	.7
C_{15} c & d	1.0	.4	.8	.2
b	2.5	1.2	2.2	.3
a	3.5	1.3	2.8	.4
C_{16} c & d	1.3	.8	.6	.2
b	11.1	4.7	17.0	2.0
a	17.6	10.7	17.4	5.8
C_{17} c & d	2.1	1.6	2.2	1.1
b	3.7	3.2	2.8	1.2
a	.9	.7	1.0	.4
C_{18} c & d	1.0	.3	.8	.1
b	16.1	16.7	9.5	6.2
a	7.4	7.4	4.2	4.3
C_{19} c, d & e	1.2	1.1	.3	.9
b	.6	.8	.4	1.4
a	.7	.9	.6	1.7
C_{20} c & d	.6	1.2	.6	.6
b	.3	.7	.3	.4
a	.6	.8	.4	1.8
C_{21} c & d	1.0	3.5	—	.1
b	1.0	1.6	10.4	23.1
a	—	—	.6	3.4
C_{22} c & d	—	1.2	.6	.8
a & b	3.9	6.6	3.6	6.8
C_{23} c	—	.7	—	—
b	—	1.1	.1	.8
a	.9	.7	.8	2.6
C_{24} c & d	—	—	.3	.1
b	.8	.5	.7	2.4
a	3.9	3.8	2.0	3.0
C_{25} c & d	.3	.5	.8	1.0
a & b	1.5	1.7	3.1	4.6
C_{26} d	1.7	8.0	2.0	7.5
c	—	—	.1	.6
b	2.0	.9	—	—
a	1.4	1.8	.7	2.9
C_{27} b & c	—	—	.6	—
a	.3	7.7	.4	6.6
C_{28} & over	1.0	2.7	2.0	2.6

tion apply to these longer chain acids. On the skin surface of normal healthy individuals relatively low amounts of these longer chain free fatty acids are present. On the other hand, of the lipids from the barrier zone of normal healthy subjects approximately one third of the free fatty acids have chain lengths longer than C_{18}. With psoriatic individuals appreciable amounts of these longer acids are present both in the surface and the barrier zone lipids. In some cases, almost three-quarters of the free fatty acids have chain lengths longer than C_{18}. There is also some indication that the nature of these acids is somewhat different in the psoriatic than in the healthy individual.

Evidence is presented which suggests that these longer acids are produced in the epidermis, at or near the barrier zone, and not by the sebaceous glands. These acids are probably incorporated into the keratin (11) or into the glycolipoprotein cementing substance (12) during the normal process of keratinization, hence only small amounts are carried to the skin surface and appear in the surface lipid film. In psoriasis, there is probably both an overproduction of these acids together with a failure of their incorporation into either the keratin or the glycolipoprotein. Hence there is an accumulation of these acids in the barrier zone and excessively large amounts are carried to the skin surface. It is not clear at this time whether this failure of incorporation is due to a defect in the mechanism of formation of the particular lipoprotein or whether the acids in themselves are so different from those normally occurring that they cannot be incorporated. In either case the results suggest some defect in lipid metabolism occurring in the psoriatic skin in the region of the barrier zone. It would be premature at this stage of the investigations to speculate on the true nature and cause of this defect. Fuller and more detailed studies of the nature of these long chain acids are at present being made, and these studies extended to other dermatoses.

SUMMARY

1. A study has been made of the composition of the free fatty acids of the lipids from the skin surface and the barrier zone of normal healthy and psoriatic subjects.

2. The presence of significant amounts of free fatty acids with chain lengths longer than C_{20} has been demonstrated particularly in the barrier zone lipids of healthy subjects and in both the surface and barrier zone of psoriatic individuals.

3. These longer chain acids appear to be pro-

TABLE II

Percentage of Acids Above C_{18} in the Free Fatty Acid of the Surface and Barrier Zone Lipids of Healthy and Psoriatic Subjects

	Healthy Normal			Psoriatic		
	No. of Subjects	Range	Mean & S.D.	No. of Subjects	Range	Mean & S.D.
Chest						
Surface.....	11	8–29*	16 ± 5	9	22–63	35 ± 12
Barrier Zone....	11	20–41	31 ± 7	9	35–69	49 ± 10
Forearm						
Surface.....	6	19–36	26 ± 6	3	31–58	46 ± 11
Barrier Zone....	6	33–52	45 ± 7	3	47–73	61 ± 11

* Results are expressed as percentages of total free fatty acids.

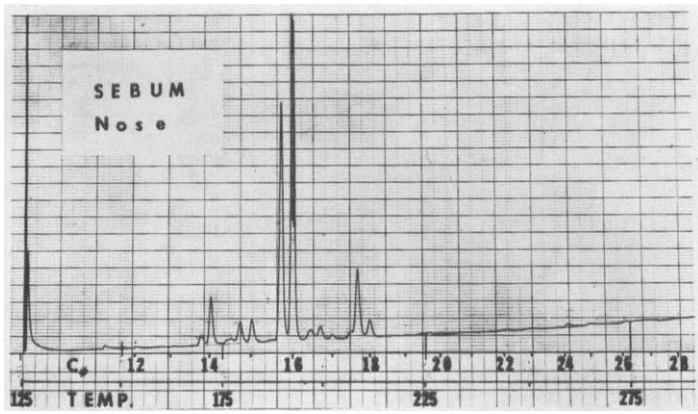

FIG. 5. Gas chromatogram of the free fatty acids from uncontaminated sebum, see text

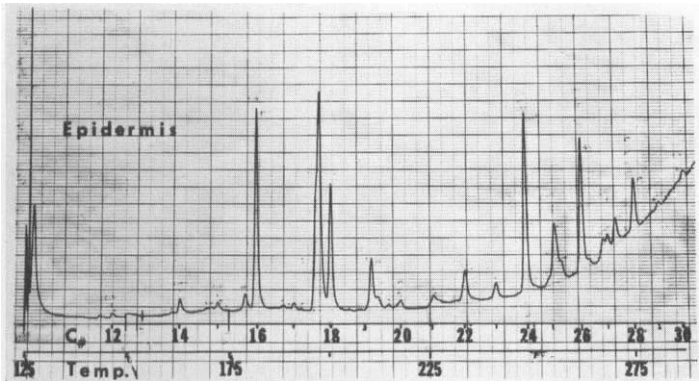

FIG. 6. Gas chromatogram of the free fatty acids isolated from the heat-separated epidermis of cadaver skin.

duced in the epidermis at or near the barrier zone and not in the sebaceous glands.

4. The significance of these findings has been discussed.

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DISCUSSION

Dr. Peter Flesch (Philadelphia, Pa): I would like to ask Dr. Coon how he obtained the barrier zone in psoriatics and whether it is true that this zone is not as well developed in the psoriatic as in the normal epidermis?

De. William M. Coon (in closing): We obtained the barrier zone, as in all our subjects, by stripping with Scotch tape. I believe in the histological studies that have been done, the appearance in psoriasis is very similar to that seen in normals.

Dr. Victor R. Wheatley (in closing): I just wish to clarify one point. These results were obtained from the uninvolved skin in psoriasis. This is not the involved skin, so I don't think Dr. Flesch's question quite applies.